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Phosphofructokinase Assay Kit

Catalog No.: abx298841

Size: 100 Assays

Storage: Store the Assay Buffer and Reaction Buffer at 4°C, and all other kit components at -20°C.

Application: For quantitative detection of Phosphofructokinase activity in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, urine, and other biological fluids.

Detection Range: 4 µmol/L – 400 µmol/L

Introduction: Phosphofructokinase is a key enzyme in glycolysis. In humans, there are two main types: Phosphofructokinase 1 phosphorylates fructose-6-phosphate to fructose-1,6-bisphosphate; and Phosphofructokinase 2 phosphorylates fructose-6-phosphate to fructose-2,6-bisphosphate. Isoforms are found in skeletal muscle, liver, and platelets. Deficiencies in phosphofructokinase can result in Tarui's disease, which is characterized by muscle weakness and cramps.

Abbexa's Phosphofructokinase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Phosphofructokinase activity in various samples. The reaction velocity is proportional to the rate of absorbance resulting from NADH reduction, which can be measured at 340 nm. The Phosphofructokinase activity can then be calculated.

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Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Reaction Buffer: 20 ml
4. Substrate: 1 vial
5. Enzyme 1: 1 vial
6. Enzyme 2: 1 vial
7. Standard: 1 vial

Materials Required But Not Provided

1. Microplate reader (340 nm)
2. Microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Mortar
6. Centrifuge and centrifuge tubes
7. Timer
8. Ice
9. Sonicator

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Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Add 17 ml of Reaction Buffer into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use.

- **Standard Solution**
  Add 1 ml of distilled water to the Standard vial and mix thoroughly. Add 200 µl of this solution to 800 µl of distilled water to prepare a 1 ml Standard Solution with concentration 400 µmol/L.

- **Enzyme 1 Solution**
  Add 1 ml of distilled water to the Enzyme 1 vial and mix thoroughly to prepare the Enzyme 1 Solution. Ensure that the Enzyme 1 has completely dissolved prior to use. Unused Enzyme 1 Solution can be stored at 4°C.

- **Enzyme 2 Solution**
  Add 1 ml of distilled water to the Enzyme 2 vial and mix thoroughly to prepare the Enzyme 2 Solution. Ensure that the Enzyme 2 has completely dissolved prior to use. Unused Enzyme 2 Solution can be stored at 4°C.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant, and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

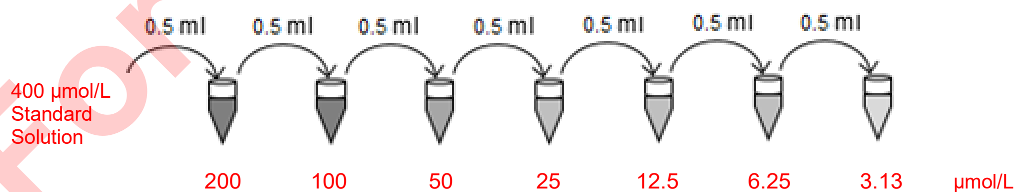
- **Serum and Plasma samples**
  Serum and plasma samples can be used directly.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected activity is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured activity within the detection range of the kit.

1. Label 8 tubes with 200 µmol/L, 100 µmol/L, 50 µmol/L, 25 µmol/L, 12.5 µmol/L, 6.25 µmol/L, and 3.13 µmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 400 µmol/L standard solution to the 1st tube (200 µmol/L) and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard, and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 200 µl of prepared standard solutions to the standard wells.
4. Add 200 µl of distilled water to the blank wells.
5. Add 10 µl of sample to the sample wells.
6. Add 170 µl of Substrate Solution to the sample wells.
7. Add 10 µl of Enzyme 1 Solution to the sample wells.
8. Add 10 µl of Enzyme 2 Solution to the sample wells.
9. Tap the plate gently to mix. Start the timer, then read and record the absorbance at 340 nm after 10 seconds and 130 seconds.

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C. Calculations

One unit of Phosphofructokinase activity is defined as the amount of enzyme required to decompose 1 μmol of NADH per minute.

Phosphofructokinase activity per mg of protein:

$$\text{PFK (U/mg)} = \frac{C_{Standard} \times V_{Standard}}{C_{Protein} \times V_{Sample} \times T} \times \frac{OD_{Sample(10s)} - OD_{Sample(130s)}}{OD_{Standard} - OD_{Blank}} = \frac{4000}{C_{Protein}} \times \frac{OD_{Sample(10s)} - OD_{Sample(130s)}}{OD_{Standard} - OD_{Blank}}$$

Phosphofructokinase activity per g of sample:

$$\text{PFK (U/g)} = \frac{C_{Standard} \times V_{Standard} \times V_{Assay}}{W \times V_{Sample} \times T} \times \frac{OD_{Sample(10s)} - OD_{Sample(130s)}}{OD_{Standard} - OD_{Blank}} = \frac{4000}{W} \times \frac{OD_{Sample(10s)} - OD_{Sample(130s)}}{OD_{Standard} - OD_{Blank}}$$

Phosphofructokinase activity per 10^4 cells or bacteria:

$$\text{PFK (U/10}^4\text{ cells)} = \frac{C_{Standard} \times V_{Standard} \times V_{Assay}}{N \times V_{Sample} \times T} \times \frac{OD_{Sample(10s)} - OD_{Sample(130s)}}{OD_{Standard} - OD_{Blank}} = \frac{4000}{N} \times \frac{OD_{Sample(10s)} - OD_{Sample(130s)}}{OD_{Standard} - OD_{Blank}}$$

Phosphofructokinase activity per ml of sample:

$$\text{PFK (U/ml)} = \frac{C_{Standard} \times V_{Standard}}{V_{Sample} \times T} \times \frac{OD_{Sample(10s)} - OD_{Sample(130s)}}{OD_{Standard} - OD_{Blank}} = 4000 \times \frac{OD_{Sample(10s)} - OD_{Sample(130s)}}{OD_{Standard} - OD_{Blank}}$$

where:

$C_{Protein}$ Concentration of protein (in mg/ml)

$C_{Standard}$ Concentration of highest standard (400 μmol/L = 400 nmol/ml)

T Reaction time (2 minutes)

W Weight of the sample (in g)

N Number of cells or bacteria (× 10^4)

V_{Assay} Volume of assay buffer (1 ml)

V_{Sample} Volume of sample (0.01 ml)

$V_{Standard}$ Volume of standard (0.2 ml)

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